Remarks

I. Support for Amendments

Support for the foregoing amendments to the claims may be found throughout the specification as originally filed, either inherently or explicitly. Hence, the foregoing amendments to the claims do not add new matter, and their entry and consideration are respectfully requested.

II. Status of the Claims

Upon entry of the foregoing amendments, claims 14-20, 27 and 32-57 are pending in the application, with claims 14, 16 and 44 being the independent claims. Claims are 14-16, 18, 27, 32, 34-37, 46, 47 and 56 are currently amended. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

III. Summary of the Office Action

In the Office Action dated March 24, 2004, the Examiner has objected to the claims, and has made three rejections of the claims. Applicants respectfully offer the following remarks to accommodate or traverse each of these elements of the Office Action.

IV. Objections to the Claims

The Examiner has objected to claim 14 for an alleged informality because the claim recites "recombination sites" in the transferring step. Office Action at page 3, section 2. Solely in an effort to facilitate prosecution, and without narrowing the scope of the claim, Applicants have amended claim 14 to recite "at least one recombination site" in the transferring step.

The Examiner has objected to claims 15 and 27 for an alleged informality because the claims recite "genomic, chromosomal." Office Action at page 3, section 3. Solely in an effort to facilitate prosecution, and without narrowing the scope of the claim, Applicants have amended claims 15 and 27 to recite "genomic DNA, chromosomal DNA."

The Examiner has objected to claim 16 for an alleged informality because the claim recites "at least a first and a second recombination site" in the inserting step. Office Action at page 3, section 4. Solely in an effort to facilitate prosecution, and without narrowing the scope of the claim, Applicants have amended claim 16 to recite "at least first and second recombination sites" in the inserting step.

The Examiner has objected to claims 34-37 for an alleged informality because the claims recite "claims 30-33," although claims 30 and 31 have been canceled. Office Action at page 3, section 5. Applicants have amended claims 34-37 to recite "claims 32 and 33."

The Examiner has objected to claims 34, 35 and 47 for an alleged informality because the claim recites "*a* eukaryotic organism," and has indicated that it should recite "*an* eukaryotic organism." Office Action at page 3, section 6. Applicants respectfully

traverse this objection. According to the common rules of grammar and usage, it is the sound of a word, not the spelling, that determines which article (*i.e.*, "a" or "an") should be used. In the case of a word that begins with a long *u* sound (*e.g.*, euphoric, European), the article "a" should be used. Since "eukaryotic" is pronounced with a long *u* sound, the use of the article "a" is proper.

The Examiner has objected to claim 44[1] for an alleged informality because the claim recites "at least a first and a second recombination site" and "at least a third and fourth recombination site." Office Action at page 3, section 7. Solely in an effort to facilitate prosecution, and without narrowing the scope of the claim, Applicants have amended claim 44 to recite "at least first and second recombination sites" and "at least third and fourth recombination sites" in steps (a) and (b), respectively.

Hence, the Objections to the claims have been rendered moot or otherwise addressed by Applicants' amendments and/or remarks, and Applicants respectfully request that the objections to the claims be reconsidered and withdrawn.

V. The Rejections under 35 U.S.C. § 112, Second Paragraph

In the Office Action at pages 4-5, sections 8-16, the Examiner has rejected claims 14, 15, 18, 32, 34, 35, 46-49 and 56 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Applicants respectfully traverse this rejection.

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[1] Applicants believe that the Examiner meant to refer to claim 44 instead of claim 16 at page 3, section 7 of the outstanding Office Action.

The Examiner contends that claim 14 is "vague and indefinite because it is unclear whether 'at least one nucleic acid molecules' in inserting step and 'one or more nucleic acid molecules' in transferring step are identical or not since the claim does not describe the relationship between [them]." Office Action at page 4, section 10. Applicants believe that the claim as originally filed, when read in light of the specification, would reasonably apprise one skilled in the art of the metes and bounds of the claimed invention. Nevertheless, without narrowing the scope of the claim, Applicants have amended claim 14 to recite: "to produce one or more integration sequence-containing nucleic molecules," and "said one or more integration sequence-containing nucleic acid molecules," thereby making explicit that which was at least implicit.

The Examiner further contends that "it is unclear that 'said nucleic acid molecule' in claim 15 means 'at least one nucleic acid molecule' or 'one or more nucleic acid molecules'." Office Action at page 4, section 11. As above, Applicants believe that the claim as originally filed, when read in light of the specification, would reasonably apprise one skilled in the art of the metes and bounds of the claimed invention. Nevertheless, without narrowing the scope of the claim, Applicants have amended claim 15 to recite: "said at least one nucleic acid molecule," thereby making explicit that which was at least implicit.

The Examiner contends that: "[s]ince claim 16 has 'at least one nucleic acid molecule' and 'a nucleic acid molecule', it is unclear that 'said nucleic acid molecule' in claim 18 means 'at least one nucleic acid molecule' or a 'a nucleic acid molecule'." Office Action at page 4, section 12. Applicants believe that the claim as originally filed,

when read in light of the specification, would reasonably apprise one skilled in the art of the metes and bounds of the claimed invention. Nevertheless, without narrowing the scope of the claim, Applicants have amended claim 16 to recite "an integration-sequence-containing nucleic acid molecule" and have amended claim 18 to recite "said integration-sequence-containing nucleic acid molecule," thereby making explicit that which was at least implicit.

The Examiner contends that there is insufficient antecedent basis for "said first and second recombination sites" in claim 32 "since there is no phrase 'first and second recombination sites' in claim 14." Office Action at page10, section 13. Applicants believe that the claim as originally filed, when read in light of the specification, would reasonably apprise one skilled in the art of the metes and bounds of the claimed invention. Nevertheless, without narrowing the scope of the claim, Applicants have amended claim 32 to recite "said at least one recombination site is a site-specific recombination site," thereby making explicit that which was at least implicit.

The Examiner contends that "it is unclear that 'said recombination sites' in claims 32 and 33 mean a first and a second recombinat[ion] sites or mean the first recombination sites or mean the second recombination sites in claims 34 and 35." Office Action at pages 4-5, section 14. Applicants believe that the claim as originally filed, when read in light of the specification, would reasonably apprise one skilled in the art of the metes and bounds of the claimed invention. Nevertheless, without narrowing the scope of the claim, Applicants have amended claims 34 and 35 to recite "said site-specific recombination sites," thereby making explicit that which was at least implicit.

The Examiner contends that "it is unclear that which above recombination sites in claim 44 [*i.e.*, first, second, third, or fourth] mean 'said recombination sites' in claims 46-49." Office Action at page 5, section 15. Applicants believe that the claim as originally filed, when read in light of the specification, would reasonably apprise one skilled in the art of the metes and bounds of the claimed invention. Nevertheless, without narrowing the scope of the claim, Applicants have amended claims 46-49 to recite "said first, second, third and fourth recombination sites," thereby making explicit that which was at least implicit.

The Examiner contends that there is insufficient antecedent basis for "said second nucleic acid comprising said first segment" in claim 56 "since there is no second nucleic acid comprising said first segment in claim 44 and a second nucleic acid comprising said first segment is not equal to the second nucleic acid recited in claim 44." Office Action at page 5, section 16. Applicants believe that the claim as originally filed, when read in light of the specification, would reasonably apprise one skilled in the art of the metes and bounds of the claimed invention. Nevertheless, without narrowing the scope of the claim, Applicants have amended claim 56 to recite "(d) selecting for said second nucleic acid molecule, wherein said second nucleic acid molecule comprises said transferred first segment," thereby making explicit that which was at least implicit.

The Examiner's grounds of rejection of claims 14, 15, 18, 32, 34, 35, 46-49 and 56 under 35 U.S.C. § 112, second paragraph, have been addressed by Applicants, and it is believed that this rejection has been fully accommodated. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, are therefore respectfully requested.

VI. The Rejection of Claims 14-20, 27, 32, 33, 44-46, and 57 under 35 U.S.C. § 102(b) Over Stemmer is Traversed

The Examiner has rejected claims 14-20, 27, 32, 33, 44-46 and 57 under 35 U.S.C. § 102(b), for allegedly being anticipated by Stemmer (U.S. Patent No. 5,605,793; Doc. AB3, of record; hereinafter "Stemmer"). Office Action at pages 6-12, section 18. Applicants respectfully traverse this rejection.

In making this rejection, the Examiner contends that:

> Since one or more double-stranded oligonucleotides comprising one or more mutations taught by Stemmer is capable of inserting randomly into a target nucleic acid molecule (ie., one or more double-stranded random fragments), one or more double-stranded oligonucleotides comprising one or more mutations taught by Stemmer is one or more integration sequences as recited in claim 14. Since one or more double-stranded oligonucleotides comprising one or more mutations taught by Stemmer are inserted into one or more double-stranded random fragments in the presence of a polymerase and form a mutagenized double-stranded polynucleotide, one or more double-stranded oligonucleotides comprising one or more mutations taught by Stemmer contain one or more recombination sites as recited in claim 14.

Office Action at pages 6-7. The Examiner uses the same language with respect to independent claim 16. Applicants respectfully disagree with these contentions.

Contrary to the Examiner's above-noted contentions, Stemmer does not disclose all of the elements of independent claims 14, 16 and/or 44 (and thus of the remaining claims that ultimately depend therefrom). In particular, Stemmer does not disclose recombination sites as recited in the present claims. As used in the present specification:

> a recombination site is a recognition sequence on a nucleic acid molecule participating in an integration/recombination reaction by recombination proteins. Recombination sites are discrete sections or segments of nucleic acid on the participating nucleic acid molecules that are recognized and bound by a site-specific recombination protein during the initial stages of integration or recombination.

Specification at pages 25-26. In contrast, Stemmer only discloses a method for performing a non-specific and non-targeted (random) sequence exchange which requires the initial restriction enzyme cleavage of the nucleic acid molecules, followed by a temperature-dependent homologous recombination via traditional crossing-over. *See* Stemmer at col. 3, lines 4-19; Example 1 at col. 11, lines 40-42 (DNAse I digestion of the DNA substrate); Example 2 at col. 12, lines 21-23; Example 3 at col. 14, lines 29-30; and Figure 2.

Furthermore, specifically with respect to independent claim 44, the Examiner contends that:

> Since 3' and 5' of the digested PCR product taught by Stemmer has restriction sites, Stemmer discloses a first nucleic acid molecule comprising at least a first segment which comprises at least a first and a second recombination site (ie., 3' and 5' restriction sites of the digested PCR product), wherein said segment comprises at least one integration sequence as recited in step (a) of claim 44. Since 5' and 3' ends of pUC18 contain restriction sites, Stemmer discloses a second nucleic acid molecule comprising at least a third and fourth recombination site (ie., 5' and 3' restriction sites of pUC18) as recited in steps (b) and (c) of claim 44.

Office Action at pages 10-11. Applicants respectfully disagree with these contentions.

The 3' and 5' restriction sites of the digested PCR product and pUC18 would not be considered "recombination sites" by one of ordinary skill as those terms are used in the present specification and claims. The presently claimed methods in which recombination proteins are involved are distinct from traditional restriction/ligation cloning methods disclosed in Stemmer.

Stemmer also fails to disclose recombination proteins according to the present invention. The Examiner contends that, "according to the definition of 'recombination

protein' in the specification, a ligase is a recombination protein and Stemmer discloses transferring one or more nucleic acid molecules formed in the inserting step comprising at [least] one recombination site into one or more vectors in the presence of one or more recombination proteins (ie., a ligase). . ." Office Action at page 7. Applicants respectfully disagree.

As made clear in the present specification, the recombination proteins that are involved in the methods of the present invention are distinct from ligases:

> Site-specific recombinases are proteins that are present in many organisms (e.g. viruses and bacteria) and have been characterized as having *both endonuclease and ligase properties*. These recombinases (along with associated proteins in some cases) recognize specific sequences of bases in DNA and *exchange the DNA segments* flanking those segments. The recombinases and associated proteins are collectively referred to as "recombination proteins" (*see, e.g.*, Landy, A., *Current Opinion in Biotechnology* 3:699-707 (1993)).

Specification at page 2, lines 3-9 (emphasis added). In contrast, the ligase enzyme disclosed in Stemmer that binds ligation sites does not have "both endonuclease and ligase properties" and does not "exchange DNA segments." Therefore, ligase, as disclosed in Stemmer, is not considered a recombination protein as that term is used in describing and claiming the present invention.

Under 35 U.S.C. § 102, a claim can only be anticipated if every element in the claim is expressly or inherently disclosed in a single prior art reference. *See Kalman v. Kimberly Clark Corp.*, 713 F.2d 760, 771 (Fed. Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984). As noted above, Stemmer does not expressly or inherently disclose the presently claimed methods. Hence, under *Kalman*, this reference cannot and does not anticipate the claims as currently presented.

In view of the foregoing remarks, reconsideration and withdrawal of the rejection of claims 14-20, 27, 32, 33, 44-46 and 57 under 35 U.S.C. § 102(b) over Stemmer are respectfully requested.

VII. The Rejection of Claims 14-20, 27, 32, 33, 44-46, and 57 under 35 U.S.C. § 102(b) Over Atlung et al. *is Traversed*

The Examiner has also rejected claims 14-20, 27, 32, 33, 44-46, and 57 under 35 U.S.C. § 102(b), for allegedly being anticipated under 35 U.S.C. § 102(b) by Atlung *et al.*, *Gene* 107: 11-17 (1991) (Doc. AT4, of record; hereinafter "Atlung"). *See* Office Action at pages 12-19, section 19. Applicants respectfully traverse this rejection.

In making this rejection, the Examiner contends that:

> Atlung *et al.*, teach to construct [sic] plasmid pTAC3599 by cloning 740-bp Taq I fragment containing the promoter appYp into the Sama I [sic] site of pTAC3575 (see Figure 1 in page 12). Since the restriction sites of the 740-bp Taq I fragment containing the promoter appYp is [sic] recombination sites, Atlung *et al.*, disclose inserting one or more integration sequences comprising at least one recombination site (ie., the 750-bp TaqI fragment containing the promoter appYp) into at least one nucleic acid molecule (ie., pTAC3575) as recited in claim 14.

Office Action at page 13. The Examiner uses the same language with respect to claim 16. *See* Office Action at page 14. With regard to claim 44, the Examiner contends that:

> Since Atlung *et al.*, teach to generate [sic] the purified BstE II-Xho I fragment of pTAC3599 carrying the phoA gene and the appYp-lacz fusion, Atlung *et al.*, disclose obtaining a first nucleic acid molecule comprising at least a first segment (ie., purified BstE II-XhoI fragment of pTAC3599 carrying the phoA gene and the appYp-lacz fusion) which comprises at least a first and a second recombination site (ie., BstE II and Xho I sites) wherein said segment comprises at lease one integration sequence as recited in step (a) of claim 44.

Office Action at page 16. Applicants respectfully disagree.

Contrary to the Examiner's contentions, Atlung does not disclose all of the elements of independent claims 14, 16 and/or 44 (and thus of the remaining claims that ultimately depend therefrom). In particular, Atlung fails to disclose integration sequences according to the present invention. The present specification states that, ". . . an integration sequence is any nucleotide sequence that is *capable of inserting randomly* into a target nucleic acid molecule." Specification at pages 22-23 (emphasis added). In contrast, Atlung discloses the cloning of nucleic acid fragments by *traditional cloning methods* into a *specific location* in a plasmid by ligating a nucleic acid molecule that has been digested with restriction enzymes into a plasmid that was digested with the same restriction enzymes to generate compatible ends. Therefore, Atlung does not disclose the use of integration sequences as that term is defined and used in the present specification and claims.

The Examiner further contends (as in the previous Office Action, *see* Paper No. 11 at page 7), that:

> Since "recombination site" was defined as a recognition sequence on a nucleic acid molecule participating in an integration/recombination reaction by "recombination proteins" (see specification, page 23, last paragraph), ligation sites (restriction sites) can be considered as "recombination sites". Since "recombinant [sic, recombination] protein" is defined as "proteins that are involved in recombination reactions involving one or more recombination sites" (see the specification, page 25, second paragraph), ligase can be considered as a recombination protein.

Office Action at pages 18-19. Applicants respectfully disagree with these contentions.

As discussed in the preceding section with regard to Stemmer, the present specification makes it quite clear that standard ligation sites (*i.e.*, sites at which nucleic

acid molecules are to be joined by ligase enzymes in traditional restriction cloning methods) are *not* considered recombination sites in accordance with the present invention:

> Site-specific recombinases are proteins that are present in many organisms (e.g. viruses and bacteria) and have been characterized as having both endonuclease and ligase properties. These recombinases (along with associated proteins in some cases) recognize specific sequences of bases in DNA and exchange the DNA segments flanking those segments. The recombinases and associated proteins are collectively referred to as "recombination proteins" (*see, e.g.*, Landy, A., *Current Opinion in Biotechnology* 3:699-707 (1993)).
>
> * * *
>
> A key feature of the recombination reactions mediated by the above-noted recombination proteins are recognition sequences, often termed "recombination sites," on the DNA molecules participating in the recombination reactions. These recombination sites are discrete sections or segments of DNA on the participating nucleic acid molecules that are recognized and bound by the recombination proteins during recombination.

Specification at page 2, lines 3-9, and page 4, lines 15-20. Hence, ligation sites (or, more accurately, restriction sites) do not qualify as "recombination sites," since the ligase enzyme that binds ligation sites (or the restriction enzyme that cleaves at restriction sites) does not "have both endonuclease and ligase properties" and does not "exchange DNA segments." This is not to say, of course, that ligation sites cannot comprise one or more recombination sites, or that recombination sites as defined in accordance with the present invention cannot be located at or near the termini of linear or nicked circular nucleic acid molecules. The point here is simply that the restriction (or ligation) sites described in Atlung cannot be considered as recombination sites as that term is defined and used in the present specification and claims. Hence, the Examiner's assertion that ligation sites

could be considered as "recombination sites" in accordance with the present invention is incorrect.

In view of the foregoing remarks and under *Kalman*, Applicants respectfully assert that Atlung cannot and does not anticipate the claims as currently presented. Reconsideration and withdrawal of the rejection of claims 14-20, 27, 32, 33, 44-46 and 57 under 35 U.S.C. § 102(b) over Atlung therefore are respectfully requested.

VIII. The Rejection of Claims 14-20, 27, 32-51 and 57 under 35 U.S.C. §§ 102 (a) or 102 (e) Over Hartley et al. *is Traversed*

The Examiner has also rejected claims 14-20, 27, 32-51 and 57 under 35 U.S.C. §§ 102(a) or 102(e), for allegedly being anticipated by Hartley *et al.* (U.S. Patent No. 5,888,732); Doc. AF3, of record; hereinafter "Hartley"). *See* Office Action at pages 12-19, section 19. Applicants respectfully traverse this rejection.

Independent claims 14 and 16 each are drawn to methods comprising, *inter alia*, inserting one or more integration sequences comprising at least one recombination site into at least one nucleic acid molecule. Independent claim 44 is drawn to a method comprising, *inter alia*, obtaining a first nucleic acid molecule comprising at least a first segment which comprises at least a first and a second recombination site, wherein said segment comprises at least one integration sequence. In contrast, Hartley does not disclose the insertion of one or more integration sequences into at least one nucleic acid molecule or a nucleic acid segment comprising at least one integration sequence.

As used in the present specification, ". . . an integration sequence is any nucleotide sequence that is *capable of inserting randomly* into a target nucleic acid

molecule." Specification at pages 22-23 (emphasis added). Thus, the Insert Donor DNA molecule disclosed in Hartley, and alleged by the Examiner to be considered an "integration sequence" (*see* Office Action at pages 20-23 and pages 25-26), cannot be considered an integration sequence as that term is defined and used in the present specification and claims.

In view of the foregoing remarks and under *Kalman*, Applicants respectfully assert that Hartley cannot and does not anticipate the claims as currently presented. Reconsideration and withdrawal of the rejection of claims 14-20, 32-51 and 57 under 35 U.S.C. §§ 102(a) or 102(e) over Hartley therefore are respectfully requested.

IX. The Rejection of Claims 14-20, 27, 32-39, 44-51 and 57 under 35 U.S.C. § 102(f) is Traversed

The Examiner has rejected claims 14-20, 27, 32-39, 44-51, and 57 under 35 U.S.C. § 102(f). Office Action at page 26. Applicants respectfully traverse this rejection.

> In making this rejection, the Examiner contends that :
>
> [Hartley et al.,] (US Patent No. 5,888,732) was filed on June 7, 1996 and published on March 30, 1999 and taught all limitations recited in claims 14, 16-20, and 30-43 [sic] (see above). However Gary Temple is not listed in [the] above patent, he can not [be] considered as [an] inventor of this instant application.

Id. Applicants respectfully disagree with the above-noted contentions.

As set forth in detail in the previous section, Hartley does not disclose all of the elements of independent claims 14, 16 and/or 44 (and thus of the remaining claims that ultimately depend therefrom). In particular, Hartley fails to disclose the use of

integration sequences. Contrary to the Examiner's assertions, the Insert Donor DNA molecule disclosed in Hartley cannot be considered an integration sequence as that term is defined and used in the present specification. In any event, the invention claimed in Hartley is not identical to that claimed in present claims 14-20, 27, 32-39, 44-51 and 57. Accordingly, that Gary Temple is not named as an inventor in Hartley is irrelevant to the propriety of his being named as an inventor in the present application. The rejection under 35 U.S.C. § 102(f), therefore, is in error.

In view of the foregoing remarks, reconsideration and withdrawal of the rejection of claims 14-20, 27-39, 44-51 and 57 under 35 U.S.C. § 102(f) are respectfully requested.

X. The Rejection Under the Judicially Created Doctrine of Obviousness-Type Double Patenting is Traversed

The Examiner has rejected claims 14-20, 27, 32-39, 44-51 and 57 under the judicially created doctrine of obviousness-type double patenting over certain claims in Hartley. Office Action at pages 26-27. Applicants respectfully traverse this rejection.

For the reasons discussed above distinguishing the presently claimed invention from the disclosure of Hartley, Applicants respectfully disagree with the Examiner's contention that the claims of the present invention are not patentably distinct from claims 27-39 of Hartley. Applicants therefore respectfully request that this rejection be reconsidered and withdrawn.

XI. Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Date: June 23, 2004

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